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Determination of Oil, Fatty Acid, Tocopherol and Antioxidant Properties of *Moringa oleifera* Seed Oil Varieties for Commercial Plantation

S. WINTHACHAI[1], P. VAITHANOMSAT[2] and V. PUNSUVON[1,3,*]

[1]Department of Chemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand
[2]Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok, Thailand
[3]Center of Excellence-Oil Palm, Kasetsart University, Bangkok, Thailand

*Corresponding author: E-mail: fscivit@ku.ac.th

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Moringa oleifera tree has never been successfully produced as the commercial plantation in Thailand due to its unavailability of high oil content varieties. Our present work reports the chemical characterization of 104 varieties of *M. oleifera* seeds imported from India and collected in Thailand. The *M. oleifera* seeds were extracted with hexane in Soxhlet extractor for oil content analysis, ranging from 25-40 % wt. The oil was further analyzed for their fatty acid composition by gas chromatography. The result showed that *M. oleifera* seed oil contained high level of unsaturated fatty acid, especially oleic acid (up to 77.12 % wt), followed by palmitic acid, stearic acid, behenic acid, eicosenoic acid and linoleic acid. Eight varieties of *M. oleifera* (40.01-41.68 % wt oil) were selected for further commercial plantation and analyzed for their tocopherol (α-tocopherol, γ-tocopherol and δ-tocopherol) content, total phenolic content and antioxidant activity.

Keywords: Fatty acid, Tocopherol, Seed oil, Antioxidant, *Moringa oleifera*.

INTRODUCTION

Moringa oleifera is in the *Moringaceae* family and *Moringa* genus[1]. It is native in Asia Minor, Africa, the Indian subcontinent (Bangladesh, India and Pakistan) and also distributed in the Philippines, Cambodia, Central America, North and South America and the Caribbean[2]. The flowers, leaves, fruits and roots are edible. In some developing countries, the powdered seeds of *M. oleifera* are traditionally utilized as a coagulant for water purification because of their strong coagulating properties for sedimentation of suspended undesired particles[3]. *M. oleifera* seed oil is commercially known as "Ben oil". The oil content ranges from 25 to 41 % and it contains high amounts of oleic acid up to 75 %, which makes it suitable for edible purposes as same as olive oil. In addition, this oil has also been used in perfume, skin lotion, illumination and lubrication, medical and cosmetic purposes and biodiesel production[4]. In Thailand, the analysis of *M. oleifera* seed oil has not been reported and it also has never been produced as the commercial plantation. In this study, we extracted *M. oleifera* seed oil imported from India and collected in Thailand. The oils were analyzed for their fatty acid composition, vitamin E (α-, γ-, δ-tocopherol), total phenolic and DPPH free radical scavenging activity. The obtained results were compared with the commercial olive oil.

EXPERIMENTAL

The 104 varieties of *Moringa oleifera* seeds were imported from India and collected from different parts of Thailand as shown in Fig. 1. The commercial olive oil was obtained from YBARRA olive oil (Sevilla, Spain). All the chemicals and reagents used in this study were from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). Folin-Ciocalteu's phenol reagent and standard 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals were from Merck (Darmstadt, Germany). Pure standards of tocopherols (α, γ, δ-tocophenrol), fatty acid methyl ester (FAME) and gallic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Extraction of *Moringa oleifera* seed oil: After removal of seed coat, the seed kernels of *M. oleifera* were crushed by chopper and dried in an oven at 105 °C for 24 h. About 3 g of grounded seeds were extracted in Soxhlet extractor (Buchi B-811, Switzerland). The oils were extracted using hexane for 2 h. After complete extraction, hexane was evaporated in a vacuum rotary evaporator and the weight of remaining oil was quantitatively measured. The extracted oils were sampled in triplicates. The oil contents were calculated following eqn. 1:

$$\text{Oil content (\%)} = \frac{\text{Weight of extracted oil (g)}}{\text{Weight of dried seed (g)}} \times 100 \quad (1)$$

Fig. 1. Seed kernel of *Moringa oleifera*

Determination of fatty acid composition: Fatty acid composition of *Moringa oleifera* oil was determined by gas chromatography (GC). The fatty acid methyl esters were analyzed by GC using the capillary column DB-WAX, 30 × 0.25 mm i.d., 0.25 μm film thickness. The temperature program was 80 °C for 2 min and then 10 °C min^{-1} up to 250 °C. Injector and FID temperatures were set at 250 °C. The carrier gas flow was helium. The internal standard used was heptadecanoic acid. The identification of the peaks was obtained by comparing their retention times with standards and the quantification was performed by the area normalization method[5].

Determination of tocopherol content: Determination of the tocopherol content was performed by high performance liquid chromatography (HPLC) with UV-visible detector. The samples were prepared as follows: 0.1 g of oil was accurately weighed into a 2 mL sample vial and dissolved in 1 mL *n*-hexane. A sample was injected into the HPLC. The analysis was carried out using a Unison UK-Silica, 150 × 4.6 mm × 3 μm of column. Detector was set at 292 nm. A mobile phase of isopropanol: *n*-hexane (0.5: 99.5, v/v) was used at the flow rate of 1.0 mL/min. A total of 20 min was enough to assay the tocopherols. Tocopherols were identified by comparing the retention times with those of pure standards of α-, γ- and δ-tocopherols and quantified on the basis of peak areas of the pure standards.

Assay of free radical (DPPH) scavenging: Scavenging of DPPH was measured according to the modified method[6]. Concisely, 10 mg of oils (diluted in ethanol) were prepared at different concentrations (10, 20, 30, 40 and 50 mg/mL). After that, 1 mL of each concentration was added into 1 mL of 0.1 mM DPPH in 95 % ethanol solution. The mixed reaction solution was kept for 0.5 h in the dark environment at room temperature and then the absorbance (A_1) was measured at 517 nm by a spectrophotometer. The scavenging rate of DPPH was calculated following eqn. 2:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \quad (2)$$

where A_0 is the absorbance of the blank reaction, containing all reagents except the oil samples and A_1 is the absorbance of oil samples.

The IC_{50} (inhibitory concentration), denoting the oil concentration required to scavenging 50 % DPPH radicals, was calculated by graphical regression analysis of the scavenging capacity *versus* oil concentrations.

Analysis of total phenolic content: The total phenolic content was analyzed by modified method[7]. Briefly explained, 125 μL of oil samples were added with 500 μL of distilled water and 125 μL of Folin-Ciocalteu reagent. The mixed reaction solution was kept for 6 min, before 1250 μL of 7 % Na_2CO_3 and 1000 μL of distilled water were added. The mixed solution was further kept for 1.5 h in the dark environment at room temperature and then the absorbance was measured at 760 nm. The total phenolic content was calculated using a graphical regression analysis of gallic acid standard *versus* absorbance. The result was expressed as mg gallic acid equivalent (GAE) per one hundred gram of oil.

RESULTS AND DISCUSSION

Oil content: The 104 varieties of *Moringa oleifera* seeds were extracted and their distribution in oil contents is shown in Table-1. The 28 samples exhibited oil content ranging from 25.01 to 30.00 % wt, while 44 and 24 samples exhibited higher oil content in the range of 30.01 to 35.00 % wt and 35.01 to 40.00 % wt, respectively. The highest oil content was observed in 8 samples ranging from 40.01 to 45.00 % wt. The variability in oil content of *M. oleifera* seeds might be due to variation in species, environmental and geological conditions of the regions[2]. The 8 varieties with highest oil content were coded as 7b-KPM, 86b-KPM, 93 K1-KPM, 105 K1-KPM, 115 K1-KPM, 119 K1-KPM, 10 K2-KPM and 11 K2-KPM. All of these varieties were selected for commercial plantation in Thailand.

TABLE-1
FREQUENCY DISTRIBUTION OF SEED OIL CONTENT FROM 104 VARIETIES OF *M. oleifera*

Oil range (% wt)	Number of samples
25.01-30.00	28
30.01-35.00	44
35.01-40.00	24
40.01-45.00	8

Fatty acid composition: The representative of *M. oleifera* seed oil chromatogram from 8 selected samples as well as olive oil was shown in Figs. 2(a) and (b). The fatty acid compositions of 8 selected samples were exhibited in Table-2. The result showed that total unsaturated fatty acid ranged from 76.26 to 81.47 % wt. Oleic acid (C18:1) (71.55-77.12 % wt) was the major unsaturated fatty acid, followed by eicosenoic acid (2.12-2.93 % wt), linoleic acid (0.58-1.52 % wt) and trace amount of linolenic acid (C18:3). Total saturated fatty acid ranged from 18.53 to 23.73 % wt. The amount of palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0) and behenic acid (C22:0) were 5.71-7.81, 3.51-6.65, 2.68-3.72 and 4.70-6.15 % wt, respectively. The fatty acid composition of *M. oleifera* seed oil was similar to that of olive oil (Fig. 2(b)) and previously reported literatures[8]. The results also showed that 84.72 % was unsaturated fatty acid whereas 15.26 % was saturated fatty acid with the major fatty acids as oleic acid

TABLE-2
FATTY ACID COMPOSITION IN 8 SELECTED OF *Moringa oleifera* SEED OIL AND OLIVE OIL

Samples	Oil content (% wt)	Fatty acid composition[a] (% wt)							SFA[b] (%)	UFA[c] (%)	O/L[d]
		($C_{16:0}$)	($C_{18:0}$)	($C_{18:1}$)	($C_{18:2}$)	($C_{20:0}$)	($C_{20:1}$)	($C_{22:0}$)			
7b-KPM	40.01	6.10	6.65	71.55	1.48	3.71	2.12	5.96	23.73	76.26	48.34
86b-KPM	41.29	6.05	6.07	73.39	0.69	3.62	2.93	5.49	22.56	77.44	106.36
93 K1-KPM	41.41	6.30	3.80	75.75	0.58	3.08	2.57	5.35	19.54	80.46	130.00
105 K1-KPM	41.15	6.41	5.88	72.03	0.85	3.72	2.72	6.15	23.23	76.77	84.74
115 K1-KPM	41.68	5.71	3.51	77.12	0.63	2.99	2.47	5.29	18.53	81.47	122.10
119 K1-KPM	41.08	6.13	4.35	75.30	1.52	2.85	2.82	5.00	19.34	80.67	49.54
10 K2-KPM	41.46	7.03	4.61	74.75	1.05	2.95	2.80	4.70	20.21	79.79	71.19
11 K2-KPM	40.53	7.81	4.22	72.88	1.10	2.68	2.37	5.05	22.35	77.64	66.25
Olive oil	-	11.00	3.32	76.05	6.77	0.41	0.31	0.12	15.26	84.72	11.23
Range[e]	40.01-41.68	5.71-7.81	3.51-6.65	71.55-7.12	0.58-1.52	2.68-3.72	2.12-2.93	4.70-6.15	18.53-23.73	76.26-81.47	48.34-130.00

[a]Row percentage of fatty acid composition might not be completely 100 % due to the non-inclusion of other fatty acids, $C_{16:0}$ is palmitic acid, $C_{18:0}$ is stearic acid, $C_{18:1}$ is oleic acid, $C_{18:2}$ is linoleic acid, $C_{20:0}$ is arachidic acid, $C_{20:1}$ is eicosenoic acid and $C_{22:0}$ is behenic acid; [b]SFA is unsaturated fatty acids; [c]UFA is saturated fatty acids; [d]Oleic acid/Linoleic acid; [e]Range values exhibited only *M. oleifera* seed oil.

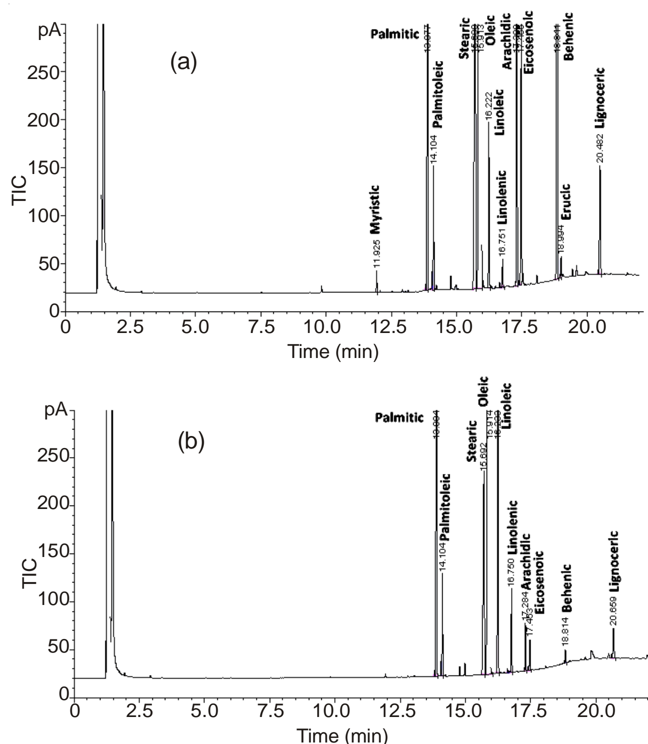

Fig. 2. GC chromatogram of fatty acid in *Moringa oleifera* seed oil (a) and olive oil (b)

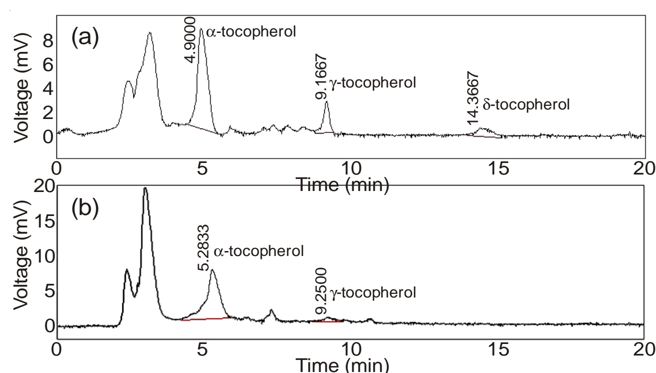

Fig. 3. HPLC chromatogram of tocopherols in *Moringa oleifera* seed oil (a) and olive oil (b)

(76.05 %), palmitic acid (11.00 %), linoleic acid (6.77 %) and stearic acid (3.32 %). The minor fatty acids were arachidic acid (0.41 %), eicosenoic acid (0.31 %) and behenic acid (0.12 %).

The oleic/linoleic acid (O/L) ratio indicates oxidative stability. Higher O/L ratio demonstrates higher stability oil. The O/L ratio of *M. oleifera* seed oil varied from 48.34 to 130.00 which was higher than that of olive oil (11.23) due to the low amount of linoleic acid in *M. oleifera* seed oil.

Tocopherol content: The HPLC chromatograms of tocopherol in the *Moringa oleifera* seed oil and olive oil were shown in Figs. 3(a) and (b). The α-, γ- and δ-tocopherol peaks appeared at 4.87, 9.00 and 14.53 min, which were the same retention times as standard peaks. The tocopherols content (α-, γ- and δ-tocopherol) was reported in Table-3. It shows that the amount of α-, γ-, δ-tocopherol and total tocopherols in

TABLE-3
TOCOPHEROLS CONTENT OF THE 8 SELECTED *Moringa oleifera* SEED OIL AND OLIVE OIL

Samples	α-Tocopherol (mg/kg)	γ-Tocopherol (mg/kg)	δ-Tocopherol (mg/kg)	Total tocopherol (mg/kg)
7b-KPM[a]	–	–	–	–
86b-KPM	162.84	80.94	10.12	253.90
93 K1-KPM	165.24	31.39	8.28	204.91
105K1-KPM	118.56	71.47	25.32	215.35
115 K1-KPM	187.18	53.19	19.99	260.36
119 K1-KPM	169.28	38.10	27.60	234.98
10 K2-KPM	189.40	111.57	14.77	315.74
11 K2-KPM	186.36	60.85	27.82	275.03
Olive oil	260.15	17.11	0.00	274.86
Range[b]	118.56-189.40	34.25-111.57	8.28-27.82	204.91-315.74

[a]There was not enough 7b-KPM sample for analysis.
[b]Range value exhibited only the *Moringa oleifera* seed oil samples.

8 samples ranged from 118.56 to 189.40, 34.25 to 111.57, 8.28 to 27.82 and 204.91 to 315.74 mg/kg, respectively.

The comparison with olive oil revealed that its α-tocopherol content (260.15 mg/kg) was higher than those in the 8 selected *M. oleifera* seed oil, whereas the content of γ-tocopherol (17.11 mg/kg) was lower than those of *M. oleifera* seed oil. Interestingly, the δ-tocopherol was not detected in olive oil. Tocopherols are regarded as one of the most valuable minor compounds present in vegetable oil because of their

antioxidant activity. Thus, the presence of antioxidant activity in *M. oleifera* seed oil was expected as in the olive oil.

Antioxidant activity: Table-4 shows total phenolic content and DPPH radical scavenging capacity of 4 selected *Moringa oleifera* seed oil (93 K1-KPM, 115 K1-KPM, 119 K1-KPM, 11 K2-KPM) and olive oil. Other four *M. oleifera* seed oil samples (7b-KPM, 86b-KPM, 10 K2-KPM, 105 K1-KPM) were not analyzed for their antioxidant activities because of their small amounts. The result showed that the total phenolic content of 119 K1-KPM was the highest at 10.30 mg GAE/100 g, follow by 115 K1-KPM, 93 K1-KPM and 11 K2-KPM at 6.56, 3.64, 2.92 mg GAE/100 g, respectively. For the olive oil, total phenolic content was 6.25 mg GAE/100 g which was lower than those in 119 K1-KPM and 115 K1-KPM but higher than those in 93 K1-KPM and 11 K2-KPM.

TABLE-4
TOTAL PHENOLICS AND DPPH RADICAL SCAVENGING CAPACITY OF 4 SELECTED *Moringa oleifera* SEED OIL AND OLIVE OIL

Samples	Total phenolic content (mg GAE/100 g)	DPPH radical scavenging (IC_{50}, mg/mL)
93 K1-KPM	3.64	43.42
115 K1-KPM	6.56	41.32
119 K1-KPM	10.30	38.17
11 K2-KPM	2.92	39.37
Olive oil	6.25	30.88

In the analysis of antioxidant activity using free radical scavenging activity of DPPH, the quantity of DPPH consumed by the antioxidant is expressed in percentage of antioxidant activity and the oil concentration required to decrease the initial concentration of DPPH 50 % is identified as IC_{50}. Therefore, the lower the IC_{50}, the higher antioxidant activity of the oil. Sample 93 K1-KPM showed 43.42 mg/mL IC_{50}, followed by 41.32 mg/mL from 115 K1-KPM, 38.17 mg/mL from 119 K1-KPM and 39.37 mg/mL from 11 K2-KPM. For the olive oil, it showed 30.88 mg/mL IC_{50}, demonstrating that olive oil had slightly higher antioxidant activity than our selected *M. oleifera* seed oil (about 13 mg/mL). This result indicated that our selected *M. oleifera* varieties have nearly property to olive oil.

Conclusion

This study presented the variation in oil content, fatty acid composition and tocopherols content from 104 varieties of *Moringa oleifera* seed oil imported from India and collected in Thailand. The *M. oleifera* seed oil contained high amount of unsaturated fatty acid, especially oleic acid (up to 77.12 % wt) and might be an acceptable substitute for highly unsaturated oils such as olive oil. Moreover, the oil had high stability. We selected 8 samples (7b-KPM, 86b-KPM, 93 K1-KPM, 105 K1-KPM, 115 K1-KPM, 119 K1-KPM, 10 K2-KPM and 11 K2-KPM), which contained highest oil content for commercial plantation. In addition, the present data on tocopherols content, total phenolic content and antioxidant activity advocated the potential use of this oil as a feedstock for the edible industries as same as olive oil industry.

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REFERENCES

1. S. Lalas and J. Tsaknis, *J. Food Compos. Anal.*, **15**, 65 (2002).
2. I.M.M. Rahman, S. Barua, M. Nazimuddin, Z.A. Begum, M.A. Rahman and H. Hasegawa, *J. Food Lipids*, **16**, 540 (2009).
3. F. Anwar and U. Rashid, *Pak. J. Bot.*, **39**, 1443 (2007).
4. M.A. Goja, *Int. J. Adv. Res.*, **1**, 181 (2013).
5. S. Panpraneecharoen, C. Khamchum, P. Vaithanomsat, M. Thanasombat and V. Punsuvon, *Annual Res. Rev. Biol.*, **4**, 2283 (2014).
6. K. Zhu, H. Zhou and H. Qian, *Process Biochem.*, **41**, 1296 (2006).
7. K. Wolfe, X. Wu and R.H. Liu, *J. Agric. Food Chem.*, **51**, 609 (2003).
8. J. Tsaknis, S. Lalas, V. Gergis, V. Dourtoglou and V. Spiliotis, *J. Agric. Food Chem.*, **47**, 4495 (1999).